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# Effect of melengestrol acetate (MGA) on cultured bovine muscle satellite cell proliferation and differentiation

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## **EFFECT OF MELENGESTROL ACETATE (MGA) ON CULTURED BOVINE MUSCLE SATELLITE CELL PROLIFERATION AND DIFFERENTIATION**

*E. K. Sissom, J. P. Kayser, A. T. Waylan, J. D. Dunn, and B. J. Johnson*

### **Summary**

Melengestrol acetate (MGA) increases growth rate and inhibits estrus in feedlot heifers. Little is known of MGA's effect on skeletal muscle growth and differentiation. The purpose of this trial was to investigate the potential direct effects of MGA on cultured bovine muscle satellite cell proliferation and differentiation. Satellite cells isolated from yearling cattle were used to assess the effect of MGA in a dose titration (0, 1 nM, 10 nM, 100 nM, 1  $\mu$ M, 10  $\mu$ M, and 100  $\mu$ M) study on [<sup>3</sup>H]-thymidine incorporation. Likewise, satellite cell cultures were allowed to differentiate, and nuclei were stained at 168 hours to determine the effect of MGA (10 nM and 100  $\mu$ M) addition during the first 48 hours on extent of differentiation and absolute myotube nuclei number. MGA addition resulted in a dose-dependent decrease ( $P < 0.05$ ) in DNA synthesis as measured by [<sup>3</sup>H]-thymidine incorporation. MGA addition (10 nM) did not significantly alter the extent of differentiation or myotube nuclei number at 168 hours in culture even though this concentration reduced DNA synthesis. However, 100  $\mu$ M MGA addition significantly ( $P < 0.05$ ) reduced both fusion percentage and myotube nuclei number as compared to control cultures. These data suggest MGA addition at concentration between 10 nM and 100  $\mu$ M affected bovine muscle cell proliferation and differentiation. A better understanding of these effects will increase our knowledge of bovine muscle growth and development.

### **Introduction**

Melengestrol acetate (MGA) is an orally active, synthetic progestogen that has been fed to feedlot heifers in the United States for over 30 years. MGA has been shown to be efficacious at increasing growth rate and inhibiting estrus in feedlot heifers. While many of the well-documented benefits of MGA have been attributed to the latter, very little information exists in relation to potential direct effects that this progestogen may have on skeletal muscle growth and metabolism, in particular to muscle satellite cell proliferation and differentiation. Satellite cells have been shown to be critically important in supporting postnatal muscle growth. Satellite cells provide the important DNA necessary to support increased muscle hypertrophy during postnatal growth. Satellite cells fuse with existing fibers and, in so doing, contribute their nuclei to the fiber. It has been estimated that approximately 60 to 90% of the DNA in a mature muscle fiber originates from satellite cells. Thus, satellite cell proliferation and subsequent fusion into muscle fibers to provide DNA required for muscle growth may be a critical rate limiting step for muscle growth. Increasing either the rate of satellite cell proliferation or the number of proliferating satellite cells could enhance the extent and efficiency of muscle growth in beef cattle. The purpose of this trial was to investigate the potential direct effects of MGA on cultured bovine muscle satellite cell proliferation and differentiation.

## Experimental Procedures

**Bovine Satellite Cell Isolation.** Satellite cell isolation was conducted using standard laboratory procedures. Cattle were sacrificed by bolting followed by exsanguination. Using sterile techniques, approximately one pound of the semimembranosus muscle was removed and transported to the cell culture laboratory. Subsequent procedures were conducted in a sterile field under a tissue culture hood. After removal of connective tissue, the muscle was passed through a sterile meat grinder. The ground muscle was incubated with 0.1% pronase in Earl's Balanced Salt Solution (EBSS) for 1 hour at 37°C with frequent mixing. Following incubation, the mixture was centrifuged at 1500 x g for 4 minutes, the pellet was suspended in phosphate buffered saline (PBS: 140 mM NaCl, 1 mM KH<sub>2</sub>PO<sub>4</sub>, 3 mM KCl, 8 mM Na<sub>2</sub>HPO<sub>4</sub>), and the suspension was centrifuged at 500 x g for 10 minutes. The supernatant was centrifuged at 1500 x g for 10 minutes to pellet the mononucleated cells. The PBS wash and differential centrifugation were repeated two more times. The resulting mononucleated cell preparation was suspended in cold (4°C) Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum (FBS) and 10% (v/v) dimethylsulfoxide (DMSO) and frozen. Cells were stored frozen in liquid nitrogen.

**[<sup>3</sup>H]Thymidine Incorporation.** Bovine satellite cells were plated for measuring thymidine incorporation. Culture plates were pre-coated with reduced growth factor basement membrane matrigel diluted 1:10 (v/v) with DMEM. Cells were plated in DMEM containing 10% FBS and incubated at 37°C, 5% CO<sub>2</sub> in a water-saturated environment. Plating density for cells was empirically established so that all cultures were 25 to 50% confluent after the incubation period. This ensured that cell proliferation rate was not affected by contact inhibition. In the first set of

experiments, 48 hours after plating the bovine satellite cells in 10% FBS/DMEM, cultures were rinsed three times with serum-free DMEM, and the appropriate levels of MGA (0, 1 nM, 10 nM, 100 nM, 1 μM, 10 μM, and 100 μM) were added in 2% bovine serum (BS)/DMEM. This range spans both the physiological and pharmacological dose of MGA *in vivo*. In the second set of experiments, appropriate MGA levels were added to the cultures immediately following the plating of the cells to give final concentrations similar to those used in the first set of experiments. At 48 hours, cultures were rinsed three times with serum-free DMEM, and 2% BS/DMEM was added. For both sets of experiments, at 72 hours, cultures were rinsed three times with serum-free DMEM and [<sup>3</sup>H]-thymidine was added to each well. Cells with [<sup>3</sup>H]-thymidine were incubated at 37°C, 5% CO<sub>2</sub> in a water-saturated environment for 3 hours. After incubation period, satellite cells were rinsed three times with cold serum-free DMEM to wash off free [<sup>3</sup>H]-thymidine. Cold 5% trichloroacetic acid (TCA) was added to every well and incubated overnight at 4°C. The following day, cells were rinsed two times with cold TCA to remove any remaining unincorporated [<sup>3</sup>H]-thymidine. The precipitated cell material was dissolved in 0.5 mL of 0.5 M NaOH by vigorously shaking the plates for 30 minutes at 37°C. The NaOH suspensions were transferred quantitatively into scintillation vials containing 10 mL of scintillation cocktail and counted in a scintillation counter. All treatments were measured in triplicate.

**Markers of differentiation.** Bovine satellite cells were plated as previously described to be used for differentiation studies. MGA (0, 10 nM, 100 μM) was added to the cultures immediately following plating of the cells. At 48 hours, cultures were rinsed three times with serum-free DMEM and 10% FBS/DMEM was added. At 96 hours, cells were rinsed three times with serum-free DMEM, and 3% horse

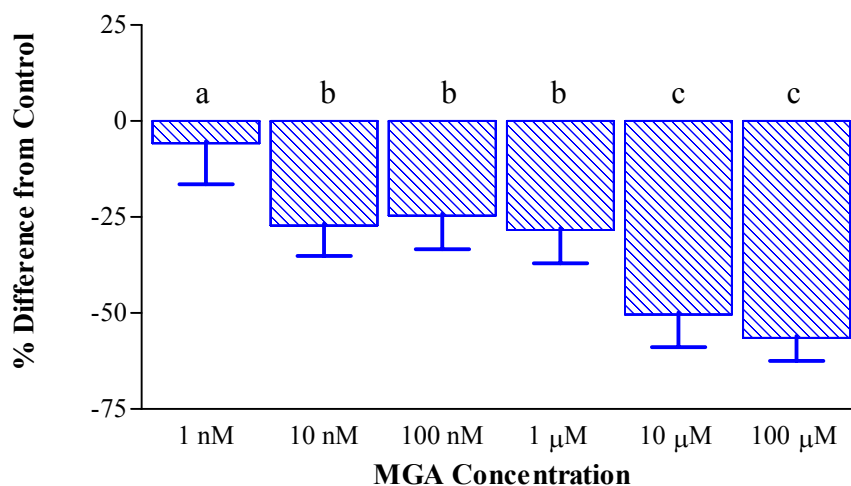
serum/1.5  $\mu\text{g/mL}$  BSA-Linoleic Acid/DMEM fusion media was added. After approximately 120 hours in culture, cells fused into multinucleated myotubes and were stained using Hoechst 33342 stain. The stained nuclei were visible under blue fluorescent light, at which time, digital photos were taken of random fields in each well to determine extent of differentiation (fusion percentage; defined as myotube nuclei/total nuclei). Treatments were measured in duplicate.

## Results and Discussion

MGA was added to bovine satellite cell cultures at concentrations that spanned 1 nM to 100  $\mu\text{M}$  (supraphysiological). Little information is available as to the blood level of MGA in heifers fed the approved dose. MGA addition to cultured bovine satellite cells resulted in a dose-dependent decrease in DNA synthesis as measured by [ $^3\text{H}$ ]-thymidine incorporation (Figure 1). [ $^3\text{H}$ ]-Thymidine incorporation of bovine satellite cells was not affected ( $P>0.05$ ) by the addition of 1 nM MGA. However, the addition of 10 nM, 100 nM, and 1  $\mu\text{M}$  MGA to cultures of proliferating bovine satellite cells reduced [ $^3\text{H}$ ]-thymidine incorporation 27, 25, and 28%, respectively, compared to the control. Additionally, MGA doses of 10 and 100  $\mu\text{M}$  further reduced [ $^3\text{H}$ ]-thymidine incorporation 50 and 57%, respectively, compared to control cultures.

The effect of MGA addition on the extent of muscle satellite cell differentiation was also assessed. Based on results of the [ $^3\text{H}$ ]-thymidine incorporation experiments, we chose 10 nM and 100  $\mu\text{M}$  MGA addition to assess these potential effects. MGA addition at a level of 10 nM did not significantly alter ( $P<0.05$ ) the extent of differentiation as estimated by fusion percentage (Table 1). Similarly, there was no difference in the absolute number of myotube nuclei when 10 nM of MGA was supplemented (Table 1). However, the addition of 100  $\mu\text{M}$  MGA significantly reduced ( $P<0.05$ ) both fusion percentage and myotube nuclei number when compared to control cultures (Table 1).

Taken together, our data suggest that MGA-supplemented cultures (10 nM) can partially overcome the MGA-dependent decrease in DNA synthesis early in the proliferation stages as demonstrated by reduction in [ $^3\text{H}$ ]-thymidine incorporation and achieve similar myotube nuclei number and fusion percentage at 168 hours. However, this was not the case for those cultures treated with supraphysiological doses (100  $\mu\text{M}$ ). Additional research investigating the mechanism of MGA at the cellular level will increase our understanding of MGA's role in improving performance of feedlot heifers.



**Figure 1. [<sup>3</sup>H]-Thymidine Incorporation of Bovine Satellite Cells Treated with Various Doses of MGA. Bars with different superscripts differ P<0.05. Control and 1 nM MGA were similar.**

**Table 1. Fusion Percentage and Myotube Nuclei in Satellite Cell Cultures Treated with MGA**

| Item                                                  | Control                 | MGA                     |                         |
|-------------------------------------------------------|-------------------------|-------------------------|-------------------------|
|                                                       |                         | 10 nM                   | 100 μM                  |
| Fusion, % <sup>a</sup>                                | 24.5 <sup>c</sup> ± 2.0 | 22.5 <sup>c</sup> ± 1.6 | 3.8 <sup>d</sup> ± 0.56 |
| Myotube nuclei <sup>b</sup> (nuclei/cm <sup>2</sup> ) | 1600 <sup>c</sup> ± 173 | 1249 <sup>c</sup> ± 112 | 159 <sup>d</sup> ± 27   |
| Total nuclei <sup>b</sup> (nuclei/cm <sup>2</sup> )   | 6488 <sup>c</sup> ± 328 | 5510 <sup>d</sup> ± 260 | 4244 <sup>e</sup> ± 289 |

<sup>a</sup>(myotube nuclei/total nuclei) x 100.

<sup>b</sup>168 hours after plating.

<sup>c,d,e</sup>Means in a row with different superscripts differ (P<0.05).